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Review

APOBECs orchestrate genomic and epigenomic editing across health and disease

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APOBEC proteins can deaminate cytosine residues in DNA and RNA. This can lead to somatic mutations, DNA breaks, RNA modifications, or DNA demethylation in a selective manner. APOBECs function in various cellular compartments and recognize different nucleic acid motifs and structures. They orchestrate a wide array of genomic and epigenomic modifications, thereby affecting various cellular functions positively or negatively, including immune editing, viral and retroelement restriction, DNA damage responses, DNA demethylation, gene expression, and tissue homeostasis. Furthermore, the cumulative increase in genomic and epigenomic editing with aging could also, at least in part, be attributed to APOBEC function. We synthesize our cumulative understanding of APOBEC activity in a unifying overview and discuss their genomic and epigenomic impact in physiological, pathological, and technological contexts.

Highlights

Genomic and epigenomic effects of apolipoprotein B mRNA editing cytosine deaminases (APOBECs) are tightly controlled and are essential for physiological immune and non-immune processes.

Pathological APOBEC off-target effects are often observed because of altered catalytic activity or dysregulation, and/or synergies with other predisposing factors such as infections, inflammation, or DNA repair defects.

APOBEC mutation signatures are documented in various cancers. They are also involved in autoimmune diseases, triple nucleotide repeat diseases, and diabetes, among others.

Possible APOBEC signatures in aging tissues also highlight their potential contribution to this process at the genomic and epigenomic levels.

Advances in gene-editing technologies combined with APOBEC mechanistic insights are ushering in the era of APOBECs as therapeutic targets, potentially closing the loop of negative versus positive gene-editing effects.

The pleiotropic roles of cytosine deaminases in genomic and epigenomic editing

Cytosine deamination can modify the genomic code by mediating cytosine to uracil (C-to-U) conversion, thus creating U:G DNA mismatches that are subsequently consolidated by error-prone DNA repair processing. Although cytosine deamination can occur spontaneously, these reactions can be catalyzed by **APOBECs** (see Glossary) in a regulated process [1–4]. Almost all APOBECs (with the exception of A3D, A2, and A4) can also edit, with varying proficiencies, the epigenomic code by deaminating **5-methylcytosine (5mC)** to thymine – creating T:G mismatches – followed by error-free DNA repair [5–9] (Figure 1, blue). Although most mutations generally have adverse effects on cells and lead to disease emergence, a few cell types evolved to harness the mutagenic process for positive physiological purposes, particularly during adaptive and innate immunity. Eleven APOBEC proteins have been described in humans. AID (activation-induced cytosine deaminase) is arguably the evolutionary founding member and has been extensively studied in the context of **somatic hypermutation (SHM)** and **class-switch recombination (CSR)** during antibody diversification [10,11]. APOBECs are also involved in several physiological processes including lipid metabolism (APOBEC1) [12], musculogenesis (APOBEC2) [13], **retroelement** restriction (APOBEC3s) [14], DNA damage (AID, APOBEC3s) [15], and cell homeostasis [16]. Despite their beneficial roles, dysregulation of APOBECs is associated with several diseases [17,18]. Signatures of APOBECs feature in many cancer genomes and are considered to be potent mediators of tumorigenesis [19,20]. Loss-of-function mutations in APOBECs can also result in immune deficiencies or autoimmunity [7,20,21]. In the following sections APOBEC family regulation (Figure 1), their genomic and epigenomic mechanisms (Figure 2), their involvement in physiology and pathology (Figure 3A–H, Key figure), and their evolutionary and technological applications (Figure 4) are synthesized and discussed from a unifying perspective.

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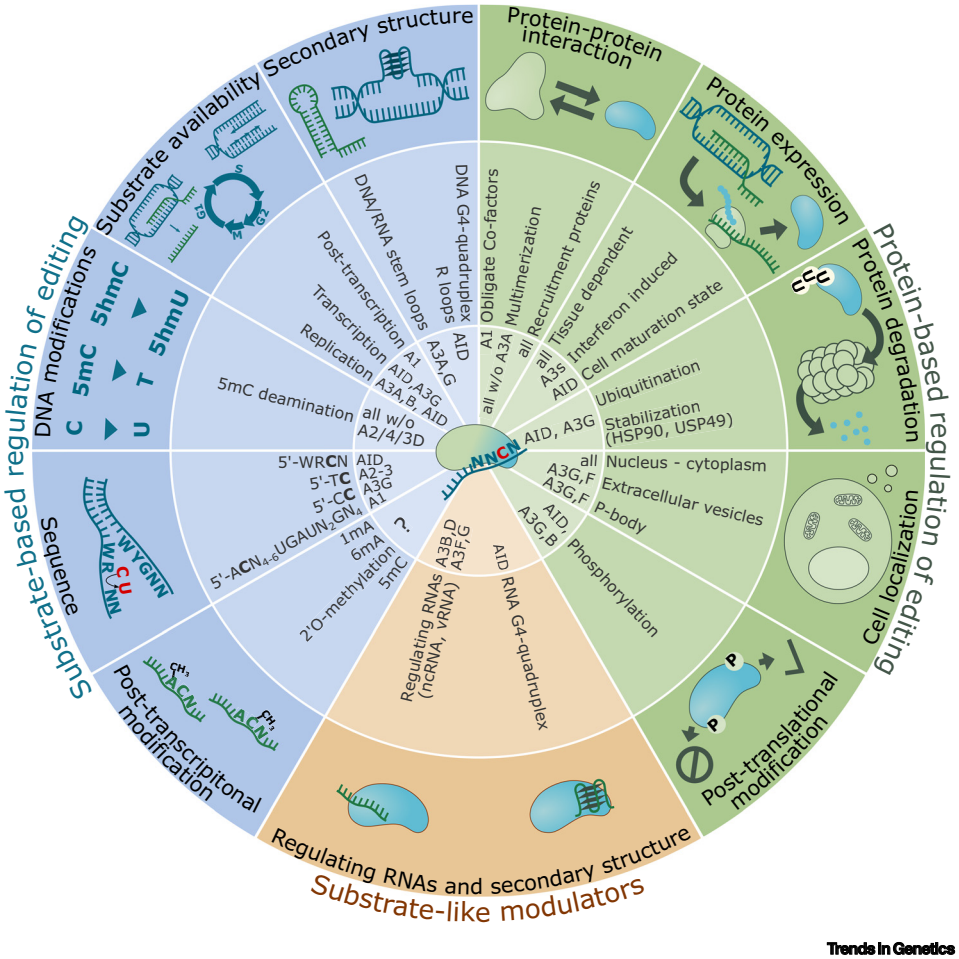

Figure 1. Protein and substrate-based regulation of APOBECs and their substrate-like modulators. The protein-based (green) activities of APOBECs can be regulated through protein expression, degradation, and cell localization. APOBECs can be further influenced by protein–protein interactions and post-translational modifications. Moreover, the properties (secondary structure, sequence, DNA and post-transcriptional modifications) and the availability of the substrate regulate the APOBEC proteins (blue). The importance of substrate-like modulators was recently discovered (orange), whereby these can bind at the catalytic center or at secondary non-catalytic domains (for A3B, A3D, A3F, A3G) to activate, inhibit, or guide the APOBEC deaminases. In the center, the dual catalytic and scaffolding functions of APOBEC are represented by blue and green shades, respectively. Abbreviations: A1, APOBEC1; A2, APOBEC2; A3A-H, APOBEC3A-H; 5hmC, 5-hydroxymethylcytosine; 5hmU, 5-hydroxymethyluracil; 1mA, N1-methyladenosine; 6mA, N6-methyladenosine; 5mC, 5-methylcytosine; ncRNA, non-coding RNA; vRNA, viral RNA. Nucleotides are depicted as N: A, T, G, or C; R: A or G; W: A or T.

APOBEC regulation: from protein domains to catalytic substrates and modulators

APOBEC catalytic activity is modulated by their ability to bind preferentially to nucleic acid substrates and motif signatures (WRC for AID and TC for most others); this mode of regulation could be considered as regulation by '*cis*-determinants'. For the APOBEC3 (A3) subfamily it has been reported that both catalytic and non-catalytic domains interact with single-stranded DNA (ssDNA) [22]. This regulates substrate deamination by guiding them to active sites in monomeric or multimeric conformations. Within the catalytic domains, positive patches distal to the active sites interact with ssDNA. In the A3F and A3G subtypes, these are reported to be crucial for binding and deamination [23]. The positively charged patches in the non-catalytic domain

Glossary

Adenosine deaminase (ADAR): edits adenosines in double-stranded (ds) RNA and DNA.

APOBECs: apolipoprotein B mRNA editing cytosine deaminase family. APOBECs include activation-induced cytidine deaminase (AID), APOBEC1 (A1), APOBEC2 (A2), APOBEC3 (A3; comprising seven subfamily members – A3A, A3B, A3C, A3D, A3F, A3G, A3H), and APOBEC4 (A4).

Class-switch recombination (CSR): a DNA recombination process in proliferating B cells that is initiated by AID deamination of intronic areas called switch (S) regions to produce antibodies with different properties. The constant region genes of the immunoglobulin heavy-chain locus rearrange and switch from one class to another.

DNA-damage response (DDR): processes by which a cell identifies, tags, and promotes the repair of DNA lesions.

DNA/RNA hybrids: hybrid double helix helices formed by one DNA and one RNA strand.

Double Double-strand breaks (DSBs): DNA lesions generated when strand breaks occur in dsDNA at nearby sites because neither base-pairing nor chromatin structure can keep the DNA ends juxtaposed.

G4 quadruplex: a DNA or RNA tetrad secondary structure that is found in guanine-rich sequences.

5-Hydroxymethylcytosine (5hmC): the oxidized form of 5mC in DNA, and a stable epigenetic mark that is crucial for gene regulation.

Kataegis: patterns of local hypermutation that are clustered in specific hotspots in the genome.

Long interspersed nuclear element 1 (LINE-1): class I transposable elements that account for 17% of the human genome; they contain two open reading frames (ORF1 and ORF2) that are required for LINE-1 retrotransposition.

5-Methylcytosine (5mC): an epigenetic hallmark that influences DNA remodeling during mammalian development and cell differentiation. 5mC is also involved in tumorigenesis.

Mismatch repair (MMR): a mechanism that identifies and corrects spontaneous insertions, deletions, and misincorporations of bases mainly generated through DNA replication, thereby maintaining genome stability.

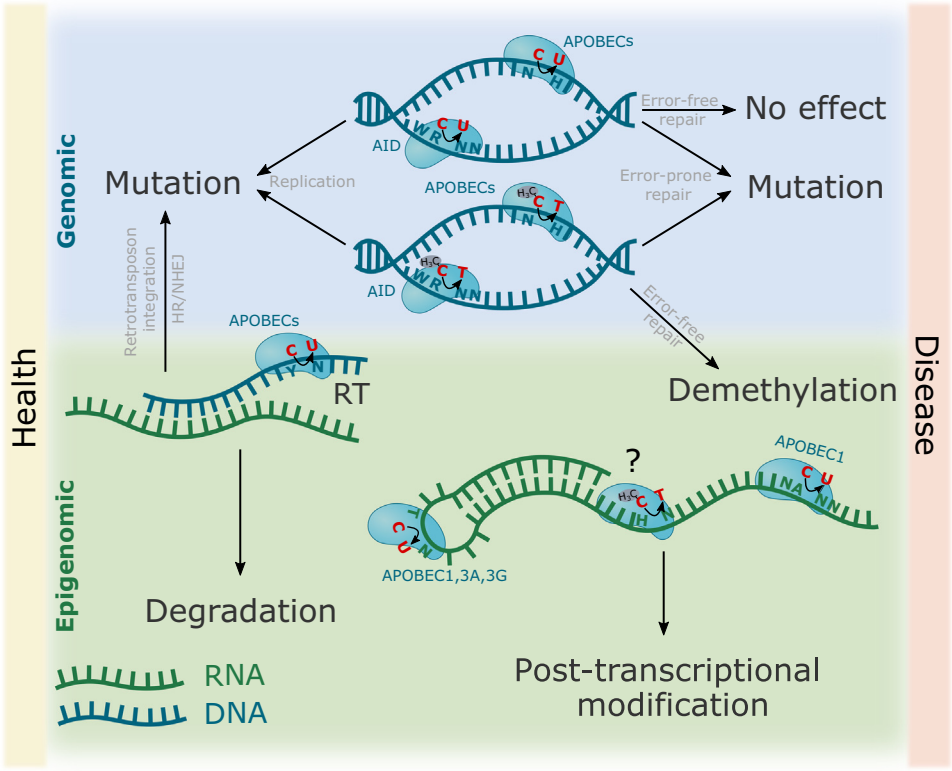

Figure 2. Genomic and epigenomic mechanisms of APOBEC deamination. APOBEC deaminases target DNA (blue) or RNA (green) substrates, leading to genetic (blue shading) or epigenetic changes (green shading). Both are known to be involved in healthy/physiological or disease/pathological processes. Abbreviations: HR, homologous recombination; NHEJ, non-homologous end joining; RT, reverse transcriptase. Nucleotides are depicted as N: A, C, G, or T; H: A, C, or T; R: A or G; W: A or T; Y: C or T.

are important to boost deamination by A3F, A3G, and A3B [22] (Figure 1). However, catalytic domains on their own have inconsistent deaminase activity, and the non-catalytic domains therefore have an important regulatory role.

APOBECs also have a preference for specific motif sequences and substrate structures. AID preferentially binds to and targets DNA **G4 quadruplex** structures during transcription [24]. G4-RNA (from transcribed switch regions) is indispensable to direct AID to its corresponding **switch-region (S)** target site. During CSR, ssDNA is formed by the **R-loop** generated through the action of RNA helicase DDX1, leading to RNA:DNA post-transcriptional events [25]. It has been suggested that AID multimerizes during CSR when interacting with G4 structures [24]. The **ribonucleoprotein (RNP)** binding proteins, HnRNP-K and -L, have been proposed as cofactors that support and regulate AID deamination during SHM and CSR [26]. The affinity of A3A is also higher for DNA and RNA hairpins [27].

By contrast, an inhibitory role has been attributed to RNA structures bound to the A3G, A3H, and AID catalytic domains. The action of RNase has long been described as being crucial for AID ssDNA deamination [28]. RNA binding promotes the dissociation of A3G multimers and restricts its deamination capacity, possibly mediated by ssDNA and ssRNA binding competition [23].

Omikli: global non-recurrent patterns of diffuse hypermutation clusters.
Processing bodies (P-bodies): stress-induced cytoplasmic RNP compartments with antiviral and potential anticancer properties.
R-loop: a triple-stranded byproduct of transcription comprising an RNA–DNA hybrid and the non-template single-stranded (ss) DNA.
Retroelements: DNA sequences that integrate into the genome after copying an RNA genome into DNA by reverse transcriptase (RT).
Ribonucleoproteins (RNP): RNA and RNA-binding protein complexes that are essential in transcription, translation, gene expression regulation, and RNA metabolism.
Single-base substitution (SBS): reference mutational signature patterns identified by the COSMIC database. There are currently about 94 such signatures, SBS1–94.
Somatic hypermutation (SHM): a process initiated by AID deamination that promotes the accumulation of mutations in the V region of the heavy and light chains of immunoglobulin genes, following by controlled selection of high-affinity antibodies in the germinal center of secondary lymphoid organs.
Switch region (S-region): intronic areas targeted by AID, uracil-DNA glycosylase (UNG), and apurinic-apyrimidinic endonuclease 1 (APE1) during CSR.
Ten-eleven translocation (TET) enzymes: methylcytosine dioxygenase enzymes that catalyze the conversion of 5mC to 5hmC in DNA; family members are TET1, TET2, TET3.
Viral infectivity factor (Vif): a viral protein found in HIV and other lentiviruses; its main function is to control the immune response by counteracting APOBECs.

Key figure

The pleiotropic role of cytosine deaminases

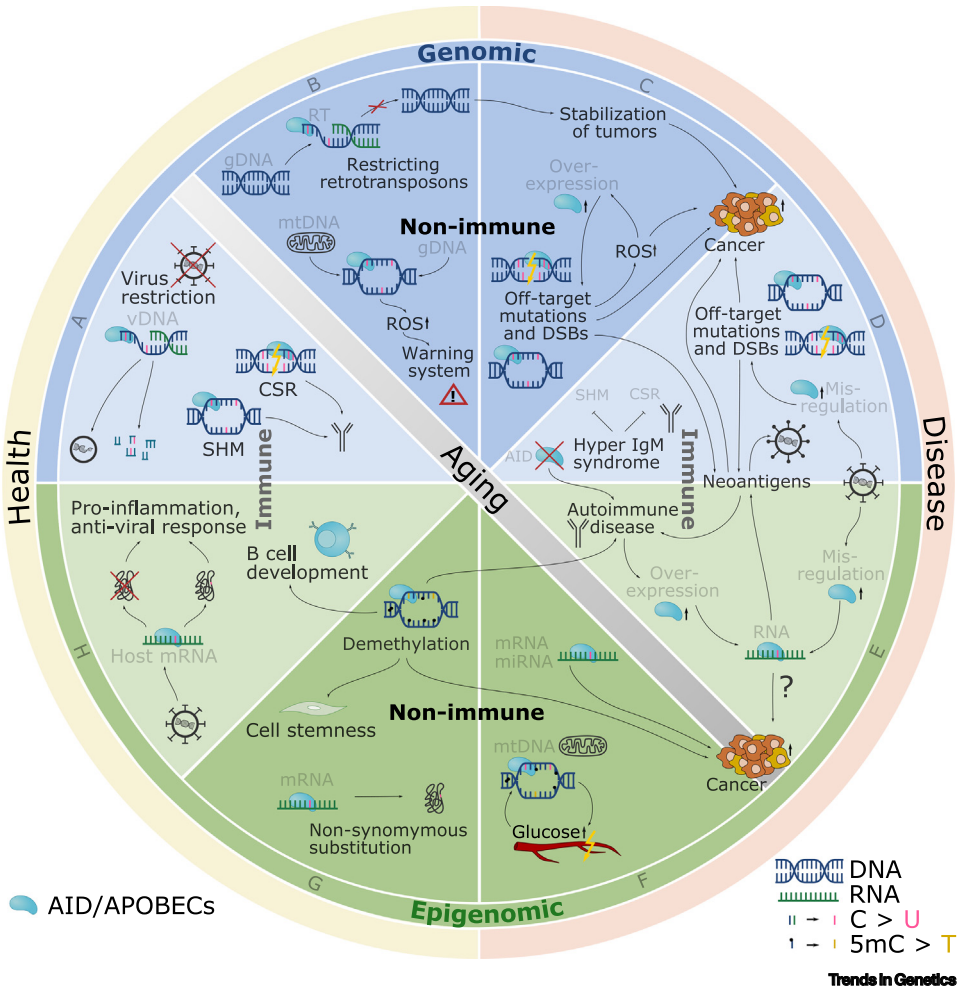

Figure 3. The APOBEC cytosine deaminase family members act at the genomic (blue) and epigenomic (green) levels. The deaminases are involved in many immune (lighter shades) and non-immune (darker shades) physiological (yellow) processes. When dysregulated, however, these enzymes can lead to various pathologies (orange). The APOBEC functions and effects are depicted in eight parts (A–H). (A) Genomic immune physiology. (B) Genomic non-immune physiology. (C) Genomic non-immune pathology. (D) Genomic immune pathology. (E) Epigenomic immune pathology. (F) Epigenomic non-immune pathology. (G) Epigenomic non-immune physiology. (H) Epigenomic immune physiology. Abbreviations: CSR, class-switch recombination; DSB, double-strand break; gDNA, genomic DNA; 5mC, 5-methylcytosine; miRNA, microRNA; mtDNA, mitochondrial DNA; ROS, reactive oxygen species; RT, reverse transcription; SHM, somatic hypermutation; vDNA, viral DNA.

RNA binding to A3H also inhibits and regulates its deaminase activity [29] (Figure 1). Notably, RNA binding to A3H loops 1 and 7 (adjacent to the A3H catalytic pocket) changes A3H structure. Residues from these loops are known to regulate 5mC deamination by A3A and A3G [30]. In A3H, it is proposed that RNA must be released from loops 1 and 7 for the enzyme to identify, bind to, and deaminate ssDNA/mC [31].

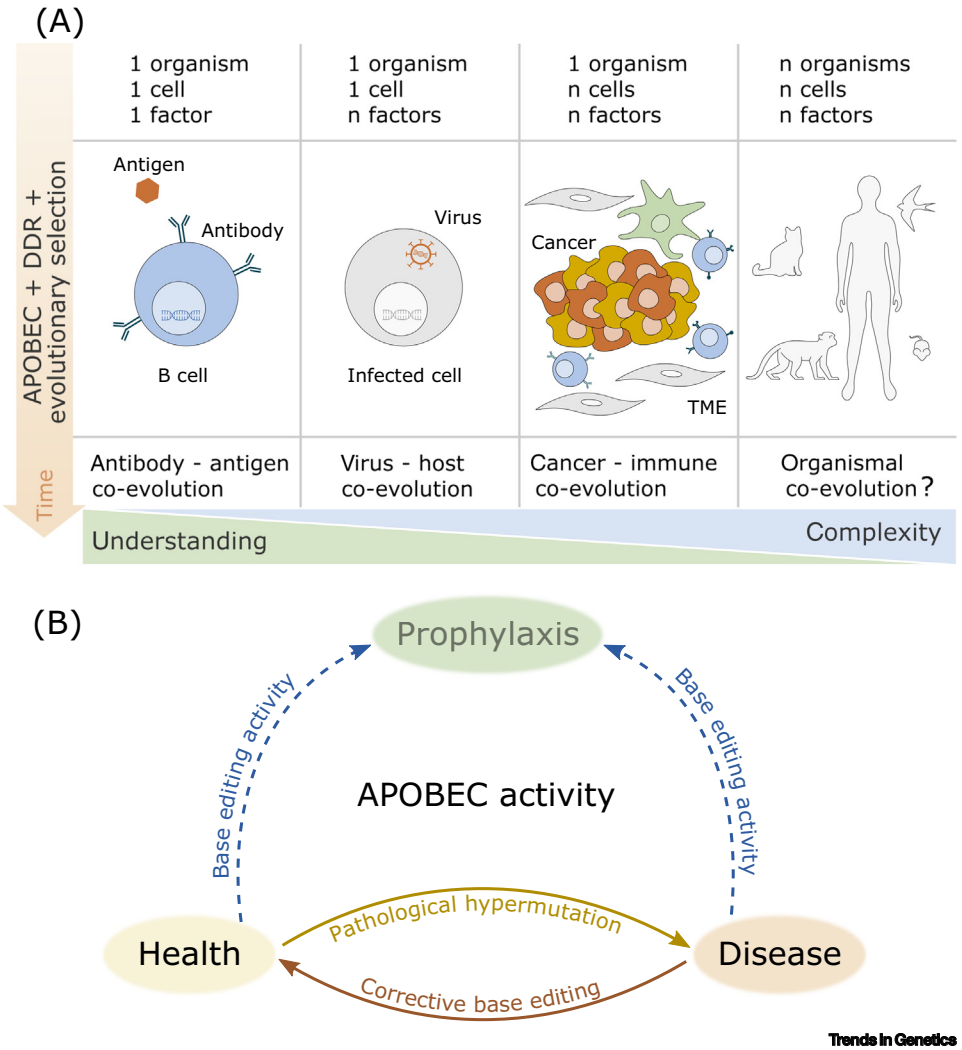

Figure 4. Evolutionarily and technologically driven gene-editing applications of APOBECs. (A) Different complexity levels of coevolution driven by APOBEC deaminases, DDR, and selective pressure over time. (B) A perspective of APOBECs as corrective and preventive tools to treat diseases at the genomic and epigenomic levels. Abbreviations: DDR, DNA damage response; TME, tumor microenvironment.

Phosphorylation is also considered to be an important APOBEC post-translational modulator. Phosphorylation of AID Ser38 residue promotes CSR; it is essential for AID and replication protein A complex 2 (RPA2) binding (Figure 1). By contrast, Thr27 phosphorylation inhibits its deaminase activity [32]. The same outcome has been described for A3B Thr214 and A3G Thr218 phosphorylation [33]. Furthermore, Thr32 phosphorylation reduces A3G affinity for HIV **viral infectivity factor (Vif)**, decreasing its degradation rate [34], highlighting the importance of the dual roles of phosphorylation in APOBECs.

APOBEC induction can also depend on '*trans*-determinants'. APOBEC often depends on environmental factors, which occur transiently throughout a lifetime, leading to the accumulation of what is now termed 'episodic' rather than consistent mutational APOBEC events [35]. Proinflammatory cytokines (IFN-α/β, TNF-α, IL-1β, and IL-6) are well-known enhancers of A3s expression.

Moreover, alternative splicing can also control APOBEC function through the generation of isoforms (e.g., A3A, B, H, and F). A3B splice variants can restrain mutagenic A3B1 expression [36]. A3H function can be diversified by alternative splicing into three isoforms: non-functional, maintained function, and enhanced viral restriction function [37].

The localization of APOBECs is another important factor that regulates their activity. They tend to be localized either within the cytoplasm or the nucleus. Thus, cytoplasmic signals (e.g., for A1, AID) or viral byproducts (e.g., for A3G) can regulate APOBEC activity. In addition, heat-shock proteins (Hsp70/90) can also stabilize AID in the cytoplasm by inhibiting its ubiquitylation and proteasomal degradation [38]. USP49 was also reported to stabilize A3G by hindering and removing the HIV Vif A3G ubiquitination mark, thereby enhancing A3G activity [39]. APOBECs can also be enclosed in P-bodies (e.g., A3G and F). A3G association in large RNP complexes suppresses its catalytic activity, but a moderate increase in A3G expression was found to decrease the formation of **processing bodies (P-bodies)** and promote stress-resistance [40]. Recent data suggest that depletion of exosome component 9 (EXOSC9; P-body formation factor) induces A3G overexpression, thus aiding APOBEC regulation positively [40].

In parallel, inhibition of **long interspersed nuclear repeat 1 (LINE-1)** and endogenous retrovirus (ERV) retroelements through G-to-A hypermutation has been long attributed to A3s (Figures 2 and 3B). Thus, the cofactors for APOBECs involved in retroelement restriction are also of interest. RPA is involved in LINE-1-retroelement integration and protects ssDNA during target-site primer reverse transcription from deamination *in vitro*, but its recent association with A3A suggests that RPA guides them both to integration sites and restricts retrotransposition [41]. A3C, A3D/E, and A3H also restrict retrotransposition, the former by creating dimers and all by interacting with LINE-1 ORF1p in an RNA-dependent manner, suggesting interplay with LINE-1 RNP [42]. In addition, extracellular vesicles secreted by cells expressing A3G and A3F can also restrict retrotransposition [43] (Figure 1, green).

Genomic and epigenomic contributions of APOBECs to immune physiology

Several APOBECs belong to the class of IFN response genes and are upregulated upon pathogen encounter in immune and non-immune cells. In adaptive and innate immunity, they act at both the genomic and epigenomic levels.

In germinal center B cells, AID is upregulated and mediates SHM and CSR of immunoglobulin genes. Thus, AID produces mutations and **double-strand breaks (DSBs)** in DNA, which enables B cells to produce functional and high-affinity antibodies against pathogens (Figures 2 and 3A) (reviewed in [44]). AID also mediates DNA demethylation and increases methylation diversity during the differentiation of naïve B cells in germinal centers [45]. Although these results indicate a contribution of AID deaminase activity, direct proof of AID involvement remains elusive. Further epigenomic changes are attributed to the APOBEC A3A and A3G, which facilitate antiviral cell responses by editing host and pathogen RNAs (Figure 3H). A3A was reported to deaminate several host mRNAs to promote proinflammatory (M1) macrophage polarization activated by viral infection, IFN, and hypoxia [46] (Figure 3H). Moreover, A3A and A3G host mRNA editing leads to altered levels of cellular proteins which are required for a successful viral life cycle [47]. However, a direct connection between the host RNA editing and the virus-restricting and proinflammatory functions of A3A remains to be fully elucidated.

APOBEC deaminases further restrict DNA viruses and retroviruses directly in an editing-dependent manner (extensively reviewed in [48]) (Figure 3A). Recently, studies on coronaviruses (CoVs) and rubella also indicated an involvement of APOBECs in RNA virus restriction. A study on

human CoV-NL63 showed inhibition of virus replication mediated by A3C, A3F, and A3H, but could not detect hypermutation of the RNA genome in *in vitro* assays [49]. However, the RNA genome of coronaviruses is highly U/A-rich [50], and mutation analysis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus shows predominant C-to-U transitions in which UCN hotspot motifs are preferentially targeted [51,52]. Interestingly, RNA viruses in invertebrates – where APOBECs are absent – do not manifest a marked increase in C-to-U mutations [53,54]. Furthermore, analysis of the RNA secondary structure elements surrounding the mutated Cs strongly indicate A3A as a potential candidate [51]. Rigorous high-resolution sequencing of SARS-CoV-2 during the pandemic provides a large number of accurate virus genome sequences that enable real-time monitoring of virus–host evolution. The contribution of APOBECs to host–virus coevolution is under investigation, and whether APOBEC-induced mutations contribute to immune escape or drug resistance of the virus has been debated (Box 1). Apart from APOBEC-mediated virus genome editing, various publications report deamination-independent restriction mechanisms, and APOBECs may therefore interfere with the replication and transcription of viral genomes by binding to viral proteins and RNAs or by blocking viral polymerases (reviewed in [48]).

Genomic and epigenomic contributions of APOBECs to immune pathology

APOBEC deaminases are also widely associated with immune-related diseases ranging from autoimmune diseases to immune cell-derived cancer and virus-associated cancers. Individuals with germline mutations in the *AICDA* gene develop a hyper-IgM syndrome (Figure 3D). They display decreased serum IgG and IgA concentrations and normal or elevated IgM levels, which subsequently lead to increased susceptibility to infections (reviewed in [55]). Furthermore,

Box 1. The contribution of APOBEC to host–virus coevolution

Viruses are predicted to have fewer APOBEC motifs in their genomes because of innate immune APOBEC deaminase editing of viral genomes over a protracted evolutionary timescale (see Figure 4A in main text). APOBECs have varying target sequence biases (see Figure 1 in main text); therefore, reduced APOBEC motif representations might differ depending on each virus. For example, γ-herpesviruses EBV and Kaposi sarcoma-associated herpesvirus (KSHV) exhibit a reduction in 5′-TC motifs that are preferentially edited by A3A/B [119]. By contrast, 5′-CCC motifs which are edited by A3G are over-represented, albeit not hypermutated [119]. Herpesvirus genomes might be protected from A3G editing because A3G (i) preferentially edits retroviruses, and (ii) is mainly located in the cytoplasm; it therefore might not be recruited and incorporated into virions. However, viruses cannot completely avoid APOBEC target motifs, especially in functionally important sequences. Therefore, some viruses have evolved proteins or miRNAs to inhibit, block, or sequester APOBEC proteins or to downregulate their expression. For instance, HIV Vif was demonstrated to mark A3 enzymes for degradation as well as to interfere with A3G transcription and translation [120]. More recently, it was shown that the EBV and KSHV relocalize A3B and A3A into cytoplasmic aggregates with their ribonucleotide reductase proteins BORF2 and ORF61, respectively, to prevent disadvantageous mutations in the viral genome (reviewed in [18]).

There is ongoing debate, especially for HIV, about whether APOBEC-induced mutations can contribute to immune escape or drug resistance of the virus. Some studies have postulated an 'all-or-nothing' phenomenon for A3G and HIV interactions, whereby therapy resistance mediated by A3G is excluded [121]. However, incomplete suppression of A3s by less potent HIV Vif mutants may enable sublethal A3 mutagenesis of the virus and might lead to advantageous viral heterogeneity [122]. An *in vivo* study investigating A3G-mediated HIV drug resistance in humanized mice showed that mice infected with HIV Vif mutants differ in their ability to counteract A3G when treated with an antiretroviral monotherapy to increase the selection pressure. However, moderate A3G activity still led to attenuation of the virus in the absence of selection, suggesting a fitness advantage in the presence of antiretroviral therapy [123]. Moreover, the analysis of all potential cytotoxic T lymphocyte (CTL) epitopes in HIV Gag, Pol, Env, and Nef showed that non-lethal A3G-mediated mutations overall result in decreased HLA binding affinities [124]. The lower affinity further leads to CTL escape rather than to more immunogenic epitopes [124]. In addition, non-lethal APOBEC-mediated editing of long terminal repeat (LTR)-retrotransposons was also proposed to be a source of host genome evolution. Integrated retrotransposons are over-represented in genes, exons, promoters, and transcription start-sites, and might be utilized for additional genomic functions [125]. In summary, years of evolution have led to a multifaceted and complex APOBEC–virus interaction which offers insights for further broader investigations into host–pathogen coevolution (see Figure 4A in main text).

patients with homozygous or heterozygous *AICDA* germline mutations are also unable to prevent the accumulation of autoreactive mature IgM B cells and their regulatory T cells show defective suppression capacity [56]. AID expression during early B cell development is important for self-tolerance and suppression of auto-IgM antibodies.

APOBEC deaminases have also shown to contribute epigenetically to autoimmune diseases such as systemic lupus erythematosus (SLE) [57] and primary Sjörgren's syndrome (SS) [58]. Epigenomic modifications play a crucial role in autoimmunity, and hypomethylation was demonstrated to increase LINE-1 endogenous retroelements in SLE and SS [59] (Figure 2). In minor salivary gland tissue from SS patients, increased LINE-1 retroelement and interferon levels were shown to correlate with elevated expression of *A3A*, *A3G*, and *AICDA* genes [58]. The same was observed in kidney tissue and peripheral blood mononuclear cells of SLE patients for A3A [58]. In addition, high-throughput sequencing data from SLE patients reveal high global APOBEC expression, which might be a source for autoantigens in SLE [21] (Figure 3E). Because some APOBECs are reported to contribute to demethylation, their upregulation might further enhance global hypomethylation in autoimmune diseases (Figure 2).

Even though AID is tightly regulated (Figure 1), and efficient repair mechanisms are in place, AID is known to contribute to B cell lymphomagenesis. Chromosomal translocations in B cell lymphomas are associated with and are partly initiated by AID-induced DSBs (reviewed in [60]). Furthermore, large-scale DNA sequencing data from lymphoid tissue of *Aicda*$^{-/-}$ or *Msh*$^{-/-}$*Ung*$^{-/-}$ mice compared to control mice revealed that 25% of the expressed genes analyzed are not fully protected from aberrant AID-mediated SHM (Figures 2 and 3D) [61,62]. Within the AID mutated non-Ig genes, many lymphoma genes including *BCL6*, *RHOH*, *PIM1*, *EBF1*, *EIF4A2*, and *PAX5* have been identified [61,62].

AID global demethylation is proposed to confer an undifferentiated state, similar to that of methylation factors in cancer, which promotes poorly differentiated tumors and low survival rates (Figures 2 and 3E–G). AID can deaminate 5mC and **5-hydoxymethylcytosine (5hmC)** (reviewed by [63]). AID demethylation of germinal center B cells cytosines is believed to exacerbate intra- and intertumor plasticity in non-Hodgkin lymphomas. Overexpression of AID in mice showed increased AID-induced methylation heterogeneity in aggressive activated B cell-like diffuse large B cell lymphomas (ABC-DLBCLs) [64]. Interestingly, epigenomic modulators are frequently mutated in DLBCL (reviewed in [60]). In chronic lymphocytic leukemia (CLL) it has also been proposed that AID demethylation correlates with worse clinical prognosis [65]. Whether AID contributes directly or indirectly to demethylation and further epigenetic changes is still under investigation. In DLBCL cell lines, it was demonstrated that AID cooperates with the **ten-eleven translocation (TET) enzyme** TET2 to demethylate the proto-oncogene, Fanconi anemia complementation group A (*FANCA*), followed by its increased expression [66]. Moreover, AID has recently been identified to display an alternative cofactor role by assisting DNA (cytosine-5)-methyltransferase 1 (DNMT1). The AID–DNMT1 complex preserves BCL6 proto-oncogene repression via promoter methylation, hence promoting negative regulation of BCL6 transcription [67].

The role of APOBEC in virus-derived cancer remains unclear and has recently become the focus of attention. It is debatable, however, whether APOBEC-mediated mutagenesis contributes to driver and/or passenger mutations. Of all cancers, 16% are estimated to be virus-associated [68] and APOBEC **single-base substitution (SBS)** mutation signatures SBS2 and SBS13 are more abundant in some virus-positive cancers than in virus-negative cancers (Figure 3D). This has been shown for human papillomavirus (HPV)-positive head

neck squamous cell carcinoma (HNSCC) which, compared to HPV-negative HNSCC, has elevated levels of APOBEC-derived mutation signatures and A3B expression [68,69]. On the one hand, it was demonstrated that driver mutations in the HNSCC oncogene *PIK3CA* are caused by A3B [69]. Nonetheless, these *PIK3CA* mutations also occur in HPV-negative HNSCC and are not exclusively linked to viral infections. In alphapapillomavirus-derived cervix and head/neck cancer the oncogenes *TP53*, *CDKN2A*, *TERT*, *FAT1* have significantly fewer driver mutations compared to virus-negative cancer [68]. In sporadic and endemic Burkitt's lymphomas, the presence of Epstein–Barr virus (EBV) was linked to an increase in the overall mutation load, and specifically in mutations attributed to AID, polymerase η (SBS9), and **mismatch repair (MMR)** failure (SBS15) [70]. Interestingly, EBV-positive compared to EBV-negative Burkitt's lymphoma harbor fewer driver mutations in B cell lymphoma-associated genes [70]. In general, this decrease in driver mutations in cancer-specific genes could be because viral proteins already fulfill key functions for tumor cell proliferation. Hence, tumorigenesis in virus-derived cancer must be considered differently and might not follow the same development as other cancers.

Genomic and epigenomic contributions of APOBECs to non-immune physiology

Physiological processes to which APOBECs contribute include hypermutating host and mitochondrial (mtDNA) genomes (Figure 3B). A3A genomic DNA deamination acts as a warning system by promoting the production of reactive oxygen species (ROS) and activating NADPH oxidases and the **DNA damage response (DDR)**. Interestingly, AID and A3s preferentially deaminate cytosines within damaged motifs, and at the same time oxidized bases feed this loop by promoting ssDNA availability [15,71]. A3A has also been shown to deaminate and hypermutate mtDNA released into the circulation by cell disruption, thus promoting catabolism and homeostasis [16,72] (Figure 3B). During cell differentiation, AID 5-methylcytosine deamination can stabilize myeloid and erythroid stem cell pluripotent phenotypes [73]. It can also maintain global demethylation and regulate cell differentiation in a deaminase-independent manner by downregulating UHRF, thus ensuring methylation maintenance during reprogramming processes [74] (Figures 2 and 3G).

The first APOBEC to be described was APOBEC1 (A1) through its ability to edit the mRNA encoding APOB protein, thereby producing an isoform involved in lipid metabolism [12,75]. However, A1 has also shown to be involve in demethylation events, for example A1 promotes nestin demethylation. *N*-methyl-D-aspartate neurotoxicity induces nestin participation in the maintenance and self-renewal of Müller cells (MCs) [76] (Figures 2 and 3G). Interestingly, under neurotoxicity, MCs in mammalian retina present a limited capacity to dedifferentiate, proliferate, and migrate to the injured regions (reviewed in [77]), suggesting A1 involvement in MC regeneration. A1 can also modulate transcriptomic diversification by editing RNAs involved in the regulation and maintenance of immune cell homeostasis, monocyte cytokine signaling/migration (e.g., RAK1/2), and maturation/phagocytosis (e.g., LAMP1/2) [78] (Figure 3G).

A3B expression was recently reported to promote cell cycle arrest by disrupting CDK4-dependent import of cyclin D1 because of a shared binding site on CDK4 [79] (Figure 1). This insight provides a novel post-translational function for A3B. A3 is also believed to negatively regulate mouse keratinocyte differentiation by inhibiting Notch3 expression; however ,the exact mechanism involved remains unknown [80].

Genomic and epigenomic contributions of APOBECs to non-immune pathology

A3s can induce genomic stress and the accumulation of mutational fingerprints that might lead to disease evolution in proinflammatory states, which in turn induce A3s and create a never-ending

loop of cell damage [71]. The deamination of A3s can also initiate ROS production, which induces their hypermutation and promotes a tumor-favorable environment (Figure 3C).

Mutational clusters in tumors, known as **kataegis** and **omikli**, have also been attributed to APOBECs [81]. Among the many DDR mechanisms, MMR activity in gene-rich regions has been recently suggested to provide ssDNA substrates for A3s and might explain the greater impact and presence of unclustered signatures of APOBECs in cancer versus conventional carcinogens (smoke, UV, etc.) [81]. Thus, by processing mismatches occurring during replication, MMR is proposed to consolidate and enhance mutagenesis of APOBECs, akin to its role in antibody diversification. DNA damage feed-forward loops generated by A3A and A3B in response to inhibition of ataxia telangiectasia and Rad3-related (ATR) protein are responsible for sensing DNA damage and activating DDR pathways [82]. In myeloma cells, A3B continuously promotes DSBs and activates DDR pathways [83] (Figure 2). DNA damage induced by conventional anticancer treatments and replication stress has been recently reported to intensify A3B expression [84]. The latter promotes a positive A3B expression loop that might lead to chemoresistant cells and promote cancer progression.

Interestingly, deamination of 5mC is known to be regulated by oxidative stress in cancer, cardiovascular diseases, and diabetes, and SOD2 overexpression was recently shown to modulate A3A activity, correlating deamination with oxidative stress [85–87]. A3A/G (C-to-U) has been recently proposed to mediate RNA-editing events that modify miRNA function in CLL [88] (Figure 2). More recently, C-to-T A3C-mediated hypermutation was reported to promote the expansion and evolution of pre-leukemic stem cells (pre-LSCs) into LSCs and secondary acute myeloid leukemia in response to an inflammatory microenvironment [20]. In epileptic patients and other neurotransmitter diseases, C-to-U A1 RNA editing also appears to be of importance because of its accumulation in glycine receptor mRNA [89] (Figure 2).

APOBECs restriction of retroelements has recently been associated with both potentiation and protection against viral infection/maintenance and with promoting carcinogenesis. Despite reports that human retroelements may play a role in carcinogenesis, these elements have also been associated with the induction of anticancer immunity – the latter through the expression of viral epitopes that can potentially drive immune responses (Box 1) [90]. Despite APOBEC protection from excessive genome instability resulting from retrotransposition, it has been proposed that APOBEC mutagenesis may generate stability in tumor cells, protect virus-infected cells, and restrict host immune responses driven by retrotransposition [91]. Retroelement activation can promote innate and adaptive immune signaling as a response to commensal colonization, pathogen infection, or cellular transformation to maintain cellular integrity [92]. Furthermore, double-stranded (ds)RNA retroelements (ERVs and LINEs) have been proposed as cytosolic RNA sensors that activate type I IFN responses [93]. Activated retroelements are also known to be a source of long non-coding RNAs that have been recently attributed a role in activating antiviral responses and as potential tumor-specific novel epitopes that promote anticancer immunity [90]. Interestingly, SETDB1, a histone H3 lysine 9 (H3K9) methyltransferase, was recently reported to help cancer cells to evade innate immune sensing of retrotransposons by inhibiting their activation [94]. Thus, retrotransposon restriction might also reflect the contribution of APOBEC to tumor development, in accord with elevated A3 activity in virus-induced cancers (discussed earlier). Interestingly, an association between retroelements and regulators of APOBECs has been reported, where repression of tumor suppressors (p53 and RB1) leads to APOBEC activation [95]. Inhibition of tumor suppressors also induces stalling of replication forks and generates ssDNA [96]. These observations suggest a possible shared pathway that might be implicated in the overexpression of APOBECs and retroelements in cancer and viral infections. A3A and

A3G RNA-editing activity can be promoted by inflammation and hypoxia [97]. A3G was reported to induce glycolysis and promote remodeling in leukemia and lymphoma cells, and also promote cancer proliferation [97]. Furthermore, A3A RNA editing was also recently suggested to modulate and amplify proinflammatory responses, and to regulate M1 polarization during viral infection and in the tumor environment [46].

A perspective on the genomic and epigenomic contributions of APOBECs to aging

The integrity of the genetic and epigenetic codes is compromised throughout the lifetime of an organism. Aging, therefore, could be broadly defined as a progressive erosion of genomic and epigenomic stability, damage accumulation, and functional decline.

MMR enhances APOBEC-mediated mutagenesis (see above), and A3 signatures were identified in essential and neurodegenerative-associated genes. Omikli events showed twice as many mutations as other age-related mutagenic events [81], suggesting that APOBECS are potentially relevant to aging-related pathologies. Furthermore, C-to-T mtDNA mutations prevail during aging and have been attributed to ssDNA availability (ROS and APOBEC substrate) and oxidative damage [98,99]. As discussed earlier, A3s can induce mtDNA hypermutation during the differentiation of neoplastic cells [100], and both share C-to-T signature biases (Figure 2). It is plausible, therefore, that these processes influence each other through aging. Moreover, the process of oxidative DNA damage removal has been tightly linked to trinucleotide repeat (TNR) expansion (Box 2) [101], and disease progression associated with aging might be explained by its synergy with APOBECs [102] (Box 2). ROS and APOBECs are known to copotentiate each other (see above), adding not only to the TNR expansion mechanism but also to age-related pathologies including cancer, whose propensity also increases with age.

The aging signatures (SBS1 and SBS5) are the largest, albeit constant, source of mutations reported in healthy tissues and cancer [19]. Close behind, APOBECs represent an important source of mutations in cancer [103]. APOBEC activity and kataegis have shown more dynamic patterns in particular conditions. Because a gradual increase in APOBEC signatures during the course of tumor evolution, and more broadly during inflammation, has been observed [20], it stands to reason that time, or age, might correlate positively with APOBEC signatures. Likewise, APOBECs are likely to contribute to late-cancer onset, progression, and tumor heterogeneity, promoting worse outcomes in older patients and potentially triggering cancerous mechanisms prior to tumorigenesis [104,105]. Inflammation, chronic or recurring, is thought to accelerate the aging process [106,107]. It is therefore conceivable that APOBEC signatures increase

Box 2. APOBEC and the trinucleotide repeat expansion mechanism

The sensitivity of APOBECs for mesoscale genomic features has been recently highlighted by their preference for hairpin structures as a substrate, including palindromic sequences which have recently been reported to show and increased propensity for mutagenesis [126]. ssDNA exposed by R-loops are also prone to APOBEC mutagenesis, and R-loop-induced deamination of cytosines in CAG repeat tracts has been deemed responsible for nucleotide repeat instability [127]. Because cotranscriptional and post-transcriptional R-loop formation can be induced and can generate mutations, genomic and epigenomic APOBEC effects necessitate tight regulation. Any dysregulation, therefore, might have implications for APOBECs in repeat expansion diseases. Pre-mRNA hairpin loops are also involved in some TNR diseases, and primary repeat motifs involved in neurological disorders display APOBEC C-to-U editing hallmarks [128]. Pre-mRNA hairpin loops are known to occur in TNR diseases; therefore, pre-mRNA might serve as a template for cDNA generation by RNA reverse transcription and lead to an expanded template to 'repair' the damage induced by APOBECs. Thus, it was proposed that APOBEC off-target mutagenesis drives Ig SHM-like RNA reverse transcription responses and is partially responsible for repeat expansion diseases [129]. Interestingly, TNR expansions have also been identified as a common feature of pan-cancer genome exon-sequencing data [130].

'episodically' following bouts of inflammation. This could potentially explain why some tissues in our body are more vulnerable to the aging process than others, notably the immune and neuronal compartments. Neurological disorders have been reported to develop simultaneously with aging. RNA editing, via A1 and its RBM47 cofactor, can maintain microglia resting status, such as RNA editing of *Lamp2* transcripts. However, when A1 is inhibited in microglia of middle-aged mice it resulted in central nervous system (CNS) proinflammation and neurodegeneration [108] (Figures 2 and 3). Interestingly, in other contexts, microglia autophagy and phagocytic/inflammatory dysfunction have also been linked to age-associated neurodegeneration [109].

The 'epigenetic clock' encoded by the DNA methylation landscape is thought to represent the most promising molecular estimator of biological age and its associated phenotypes across various species [110]. These epigenetic instabilities lead to damage accumulation, and can be induced by environmental factors or errors in epigenetic transmission mechanisms or deregulation of methylation maintenance mechanisms, such as DNMTs and TETs, thereby generating heterogeneous methylomes among the elderly [111]. Because global hypomethylation of CpG islands is another shared feature between aging and cancer, and because APOBECs can demethylate (via deamination) both epigenetic marks (5mC and 5hmC), it would be interesting in the future to study the association between APOBEC activity/signatures and aging or aging-related phenotypes, genetically and/or epigenetically (Figure 2). It would be tempting to speculate, therefore, that aging might be an undesirable, albeit unavoidable, side-effect of antibody–antigen, host–pathogen, and immune–tumor editing coevolution (Figure 4A).

Concluding remarks

In summary, APOBECs fulfill complex positive and negative roles (Figure 3) across fundamental evolutionary processes through shared molecular mechanisms (Figure 4A). Following this trajectory of research, it might be plausible to extrapolate an even broader role for APOBECs in

Box 3. Base editors in treatment and therapy

Single-nucleotide variants (SNVs) represent the majority of human disease-related genetic polymorphisms [131]. Correction of pathogenic SNVs requires high-precision genome-engineering technologies. Since the discovery of the prokaryotic-derived clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated 9 (Cas9) system it is possible to introduce targeted DNA DSBs at any given site. However, through error-prone non-homologous end joining (NHEJ), non-specific insertions and deletions (indels) alter the gene sequence randomly [132]. That lack of accuracy makes standard CRISPR/Cas9 unsuitable for precise editing. To overcome this limitation base editors (BEs) have emerged. Cytosine base editors (CBEs) are a hybrid system merging the benefits of prokaryotic and mammalian adaptive immunity [133–136]. By linking a nuclease-deficient Cas9 to a catalytically active cytosine deaminase, BEs can create precise point mutations without causing unwanted DSBs or indels [131]. Within a narrow editing window of about 5 nt the target C is changed to a T. The DNA repair inhibitor uracil glycosylase inhibitor (UGI) is often included to enhance C-to-T transition. A variety of different wild-type and modified cytosine deaminases, including APOBEC members and AID, have been studied (reviewed in [137]). In addition to CBEs, adenosine base editors (ABEs) enable genomic A-to-T mutations. ABEs use the engineered *E. coli*-derived tRNA **adenosine deaminase** [137]. Dual BEs and transversion CBEs are further developments that enable simultaneous C-to-T/A-to-G and C-to-G mutations, respectively [138,139]. Engineering of the Cas9 domain or replacement by other variants broadens their applicability [140].

The therapeutic potential of CBEs and ABEs has been reported in different genetic disorders (see Figure 4B in main text). CBEs efficiently reversed T-to-C point mutations *in vivo* in genes that drive phenylketonuria, recessive hearing loss, β-thalassemia, and Marfan syndrome, respectively [141–144]. Impressively, one-time injection of an ABE into mice with Hutchinson–Gilford progeria was sufficient for complete disease remission [145]. Instead of correcting pathologic SNVs, BE can also insert point mutations into promoter regions to alter gene expression. That strategy has been successfully applied in sickle cell disease to downregulate the BCL11A repressor protein [146]. BE-induced nonsense mutations can further disrupt disease-related genes [147]. An indirect therapeutic benefit of BEs was shown for cancer immunotherapy by improving multiplexed immune cell engineering [140]. Overall, BEs demonstrate that AID and APOBECs can be applied in a programmable fashion to reverse pathogenic point mutations or alter the expression of disease-related genes (see Figure 4B in main text).

Outstanding questions

Do chronic and acute infections regulate APOBECs activity in a different manner? If chronic infections contribute to persistent low APOBEC activity and the accumulation of hypermutations, does this contribute to cancer development depending on additional environmental factors? If acute infections occur in cancer patients, does this potentiate APOBECs hypermutation activity and lead to cell death, thus restricting cancer development?

Is APOBEC-associated genomic/epigenomic editing a driver or passenger in tumorigenesis? Do APOBECs contribute to tumorigenesis and tumor immunity positively, negatively, or stochastically? What is the synergistic contribution of DDR in this process?

How does the epigenomic landscape affect APOBEC activities, and what feedback loops are involved? Can APOBECs edit methyl-C in RNA?

Do APOBECs fulfill synergistic or antagonistic functions with other deaminases in aging and tumor immunity?

Do APOBECs contribute to organismal diversification in a similar manner to their role in immune and cancer diversification (Figure 3A). If so, what organism(s) could serve as a model.

Can gene editing through APOBECs provide future avenues for corrective or prophylactic interventions against debilitating diseases and aging? Are APOBECs a good therapeutic target for small-molecule inhibitors or through APOBEC activity mimicry? (Figure 3B).

organismal coevolution (Figure 4B), and a few studies have suggested such a possibility [5]. It is important that we understand the intricate molecular differences between the positive and negative outcomes of editing such that we could selectively harness the former and limit the latter. For example, APOBEC A3B hypermutation has been associated with better outcomes in ovarian carcinoma [112]. In addition, A3B and A3A hypermutated breast cancer recently reported similar positive clinical outcomes compared to less mutated cancer [113]. This unexpected feature might be explained by an incremental increase in the neoantigenic load, thereby increasing anticancer immunity [114]. For this reason it was recently proposed that APOBECs may play a role as a sensitizing system to improve chemotherapeutic treatments [115] (Figure 3). This novel therapeutic approach relies on actively driving mutations to generate novel neoepitopes presented by tumor cells and thus prime antitumor T cells to make tumors susceptible to immunotherapy [116]. A3B has also been postulated as a potential target for future therapeutic approaches [117]. A3B is known to be upregulated by temozolomide, a common chemotherapy in glioblastoma [118]. Estrogen was recently reported to sensitize GBM cells to temozolomide chemotherapy through estrogen receptor β (ER-β) by modulating DDR signaling pathways and diminishing DNA repair, leading to apoptosis [117]. These events might be complemented and explained by the action of A3B (Figure 3). The beneficial and detrimental features of APOBECs thus place them as precise editors and therapeutic targets (Figure 4B). For this reason there is an urgent need to better understand their regulation and mechanisms of action to enhance their biotechnological applications (Box 3 and see Outstanding questions).

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Declaration of interests

The authors declare no conflicts of interest.

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